Radiation Protection Dosimetry (2014), Vol. 159, No. 1–4, pp. 46–51
Advance Access publication 21 April 2014
doi:10.1093/rpd/ncu127

FURTHER BIODOSIMETRY INVESTIGATIONS USING MURINE PARTIAL-BODY IRRADIATION MODEL

W. F. Blakely[1,*], D. J. Sandgren[1], V. Nagy[1], S.-Y. Kim[1], G. B. Sigal[2] and N. I. Ossetrova[1]
[1]Armed Forces Radiobiology Research Institute, 8901 Wisconsin Avenue, Bethesda, MD 20889-5603, USA
[2]Meso Scale Diagnostics, Rockville, MD 20850, USA

*Corresponding author: william.blakely@usuhs.edu

This study evaluates both the effects of physical restraint and use of candidate biomarkers in a CD2F1 male mouse partial-body irradiation model for biological dosimetry diagnostic assays. Mice were irradiated (6-Gy, 250-kVp X ray) to 3/3rd (total body), 2/3rd (gut and torso), 1/3rd (gut only) and 0/3rd (sham) of total body. Blood was sampled for haematology and blood plasma proteomic biomarkers at 1 and 2 d after exposure. Increases in the body fraction exposed showed progressive decreases in lymphocyte counts and increases in the neutrophil-to-lymphocyte ratios with no significant differences in the neutrophil and platelet counts. The radioresponse for plasma biomarker Flt3L showed proportional increases; however, G-CSF and SAA levels exhibited dramatic and non-proportional increases in levels. Physical restraint at 1 d post-exposure increased lymphocyte counts and SAA, decreased neutrophil-to-lymphocyte ratio and Flt3L and showed no effects on neutrophil and platelet counts or G-CSF.

INTRODUCTION

Radiation accidents typically involve non-homogenous partial-body irradiation (PBI) exposures, while studies to establish dose–response calibration curves for biodosimetry applications generally use total-body irradiation (TBI) models[1]. Radiation models need to be developed to assess confounders including PBI exposure. There are extensive studies evaluating the effects of partial-body exposures using cytogenetic radiation biomarkers[2]. A recent study was reported evaluating multiple parameter biodosimeters using a total- and partial-body baboon radiation model[3]. The authors previously reported preliminary results to establish a murine PBI exposure model and to evaluate the effects of PBI vs. TBI exposures on radiation-responsive haematological and plasma proteomic biomarkers[4].

Strategies for medical management of a suspected radiation overexposed individual are enhanced with an understanding of fraction of the body exposed as well as an assessment of radiation injury for organ-specific systems. Biomarkers can fall into two classes, early expressed biomarkers of radiation injury as well as organ-specific injury biomarkers that are exhibited at various times after radiation exposure in a time- and dose-dependent fashion based on organ- and tissue-specific cell-renewal transit times[5]. Biomarkers for the assessment of the severity of injury due to various acute radiation sickness (ARS) subsyndromes have been identified, validated in animal model systems and used in radiation therapy and accidents; see review by Blakely *et al.*[6].

In the murine PBI model for biodosimetry evaluation, mice were immobilized by physical restraint during the irradiation. Stresses from various sources have the potential to confound the evaluation of radiation dose by biological methods[7]. Immobilization stress can alter the prooxidant–antioxidant balance and differed in radioresistant vs. radiosensitive rats[8].

Here the aim of this study was to establish a murine PBI model to evaluate use of early-phase bioindicators of severe radiation injury that have the potential to enhance ability to rapidly identify severely exposed individuals. The authors performed a multiple parameter biodosimetry study using mice exposed to progressive increases in body fractions with 6 Gy of 250 kVp X rays. Herein, the authors extended their findings to include an assessment of the effects of stress due to physical restraint used to immobilize mice during partial-body exposures on candidate radiation biomarkers. The utility of using multiple stress-resistant radiation biomarkers to assess partial-body exposure is shown.

MATERIALS AND METHODS

Animal model system

CD2F1 mice (*Mus musculus*), 8- to 10-week-old (~22–26 g) males were used in this study. Studies were performed in a facility fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International and treated in accordance with principles outlined in the *Guide for the Care and Use of Laboratory Animals* of the Institute of Laboratory Animal Resources, National Research Council as previously described[9, 10].

Radiation exposure and dosimetry

Mice were exposed to 0 or 6 Gy of X rays either in mouse radiation boxes (Figure 1A) or single-mouse Plexiglas® jigs (Figure 1B). Immobilized mice exposed

Report Documentation Page

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
21 APR 2014		**00-00-2014 to 00-00-2014**

4. TITLE AND SUBTITLE	5a. CONTRACT NUMBER
Further Biodosimetry Investigations Using Murine Partial-body Irradiation Model	5b. GRANT NUMBER
	5c. PROGRAM ELEMENT NUMBER
6. AUTHOR(S)	5d. PROJECT NUMBER
	5e. TASK NUMBER
	5f. WORK UNIT NUMBER
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) **Armed Forces Radiobiology Research Institute,8901Wisconsin Avenue,Bethesda,MD,20889**	8. PERFORMING ORGANIZATION REPORT NUMBER
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)	10. SPONSOR/MONITOR'S ACRONYM(S)
	11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION/AVAILABILITY STATEMENT
Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES
Radiation Protection Dosimetry (2014), Vol. 159, No. 1-4, pp. 46-51

14. ABSTRACT

15. SUBJECT TERMS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT **unclassified**	b. ABSTRACT **unclassified**	c. THIS PAGE **unclassified**	**Same as Report (SAR)**	**7**	

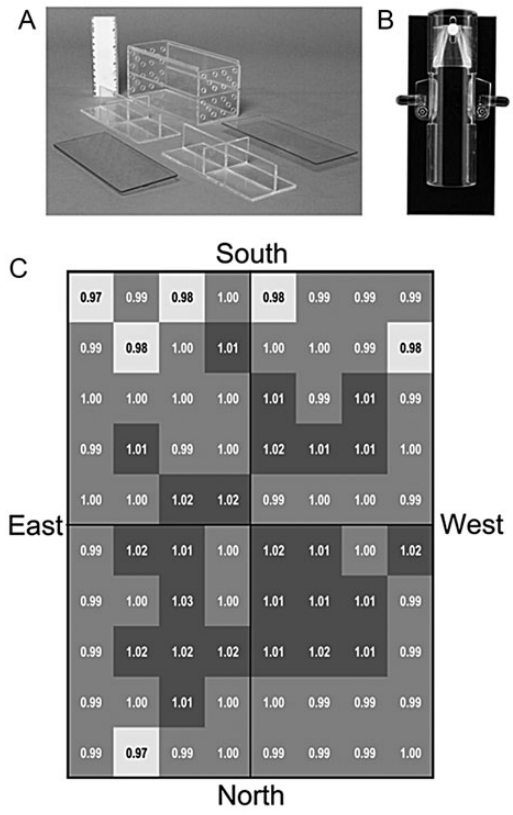

Figure 1. Apparatuses for holding mice during irradiation and dosimetry mapping for irradiating non-restrained mice. (**A**) Photograph illustrating mice Plexiglas® irradiation box apparatus used for irradiation of non-restrained mice. Typically four mice were placed in the bottom of the mouse irradiation box. (**B**) Photograph illustrating single-mouse Plexiglas® holder used to restrain mice in the partial-body studies. (**C**) Dosimetry mapping in the field of the mouse irradiation box used for irradiating non-restrained mice to X rays. Dose uniformity mappings shown as the ratio of doses relative to the measured mid-line dose of 6 Gy using alanine EPR dosimetry. The grayscale key is also illustrated for the dosimetry mapping (see manuscript text for additional details).

to X rays in the restrained condition were either sham treated (0/3) or exposed to 6 Gy of X rays representing increasing fractions of the total body: 1/3 (mid-body), 2/3 (mid- and lower-body) and 3/3 (whole-body). The PBI setup and dosimetry was earlier described[4]. Three replicate experiments were performed with $n = 8$ animals for each group for each experiment.

Mice were irradiated with a Philips X-ray machine (250 kVp, filament current 12.5 mA, HVL 2.1 mm Cu, SSD = 50 cm) at a dose rate at the cores of the mouse abdomens ~0.5 Gy/min. The doses and dose uniformity for the X-ray exposures using non-restrained mice were measured as previously described[4].

Peripheral blood biosampling and blood cell counts

Peripheral blood biosampling was performed in this study as previously described[4]. Briefly, blood was drawn from mice under anaesthesia by cardiac puncture using heparin wetted needles (23 G) and 1 ml syringes. The blood was then transferred into EDTA vacutainer tubes (Becton Dickerson & Company, Franklin Lakes, NJ, USA) and analysed within hours after biosampling. Complete blood cell counts and differentials were determined using a clinical haematology analyser.

Plasma protein biomarker measurements

A panel of candidate radiation-responsive plasma protein biomarkers were measured using the Meso Scale Diagnostic (MSD) MULTI-ARRAY® electrochemiluminescence-detection technology, which exhibits high sensitivity and dynamic range capabilities[11]. Assays were developed as multiplex panels in 16-spot MULTI-ARRAY 96-well plates and analysed on an MSD PR2 Model 1800 plate reader as previously described[12].

Selected plasma protein biomarkers (i.e. Flt3L, SAA, G-CSF) were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits (Flt3L and G-CSF: R&D Systems, Minneapolis, MN, USA; SAA: Invitrogen Corporation, Camarillo, CA, USA) as previously described[9, 12]. Briefly, samples were assayed for colorimetric detection and quantitation of total protein via the bicinchoninic acid method (Pierce, Rockford, IL, USA) prior to the immunoassay. Three replicate measurements were determined for each sample and standards. The Flt3L, SAA and G-CSF concentrations in plasma samples were determined via the generated calibration curve for standard proteins and with use of Table Curve 2D software (Systat Software Inc., San Jose, CA, USA).

Data analysis

The analysis of variance was used when comparing more than two groups and two-sided Student's *t*-test was used when comparing two groups to determine significant difference among sampling time and dose points. Values of $p < 0.05$ were considered statistically significant. Values are expressed as mean ± standard error (SE).

RESULTS AND DISCUSSION

Current methodologies to rapidly assess PBI exposure and radiation injury need to be enhanced to provide necessary diagnostic information to support early-phase medical treatment decisions[13]. A mouse PBI

model was established to evaluate candidate early-phase radiation bioindicators. Preliminary findings addressing the effects of partial-body exposure to selected biomarkers were earlier reported[4]. Here, the authors present results on the effects of restraint on candidate biomarkers and expand upon the panel of biomarkers evaluated in the earlier partial-body exposure study.

Dose uniformity

The uniformity of the dose was determined using EPR dosimetry with alanine pellets positioned in the boxes at the location to be occupied by the animals (Figure 1A). Dose resolution is limited by the diameter (5 mm) and spacing (~2–3 mm) between alanine pellet. Results for irradiation of restrained mice were previously described[4]. Based on this resolution the uniformity of the field was within 2–3 % for the non-restrained mice exposed to 6-Gy X rays in the mouse radiation box (Figure 1C).

Plasma radiation biomarker profiling

Plasma derived from non-restrained 0 and 6 Gy of X-ray exposed mice was used to screen for candidate radiation-responsive biomarkers measured by the MSD detection technology. Results from individual animals for multiple candidate plasma biomarkers are shown in Figure 2 and illustrate the interindividual variations in baseline levels (0 Gy) compared with irradiated (6 Gy) mice 1 and 2 d after exposure. The radioresponse for IL-6 and SAA, shown here for mouse strain CD2F1, is similar to the results the authors reported earlier using BALB/c male mice[9]. Many of the plasma proteins exhibited low to negligible radiation effects, whereas others (Flt-3L, SAA and G-CSF) showed promise as useful diagnostic radiation biomarkers. These three plasma protein biomarkers were evaluated in subsequent studies evaluating the effects of partial-body exposure and restraint.

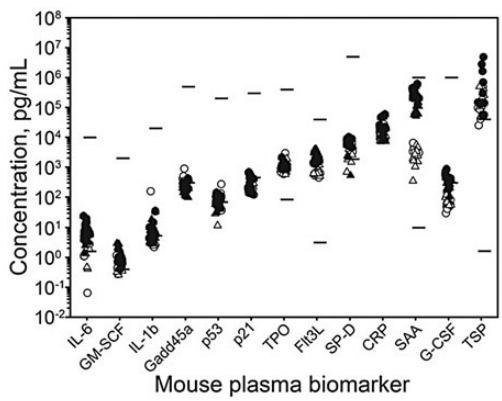

Figure 2. Effect of irradiation to 0 and 6 Gy X rays on plasma protein biomarkers in non-restrained mice. Symbols represent various plasma protein biomarker levels from individual mice 1 d (○, •) and 2 d (Δ, ▼) after exposure to 0 Gy (○, Δ) and 6 Gy (•, ▼) X rays. Mouse plasma proteins were measured using MSD detection technologies even if concentrations was less than LOD. Line bars illustrate the upper and lower assay detection limits in the linear range.

Effect of restraint

Radio-responses of biomarkers can be influenced by many confounders including co-exposure to wounding, blast or overpressure, heat or burns, medication and partial-body exposures. The PBI mouse model allowed to evaluate the stress effects from restraint during irradiation. Mice were immobilized during the partial-body exposures of ~12 min duration using single-mouse holders (Figure 1B) as previously described[4] in lieu of anaesthesia. In order to evaluate the impact of restraint, a cohort of mice were also exposed to 0 and 6 Gy of X rays in mouse radiation boxes (Figure 1A) representing the non-restrained condition.

Results from selected haematology parameters (lymphocytes—Figure 3A; neutrophils—Figure 3B; neutrophil-to-lymphocyte ratio—Figure 3C; platelets—Figure 3D) and plasma protein biomarkers (Flt3L—Figure 3E; SAA—Figure 3F; G-CSF—Figure 3G) are shown. Baseline data from mice taken directly from their normal cages are shown as a horizontal line in Figure 3A–G. These levels are similar to values obtained from control (0 Gy) animals in the restrained and non-restrained conditions.

Fold changes in biomarker levels comparing restrained vs. non-restrained conditions are shown in Table 1. Restraint induced an ~3 fold ($p \leq 0.001$) increase in SAA levels 1 day after 6 Gy of X-ray exposure. Lymphocyte counts were elevated by ~1.8-fold ($p = 0.003$), whereas neutrophils-to-lymphocytes ratio decreased by 0.6- to 0.7-fold ($p \leq 0.02$) 1 and 2 d after 6 Gy of X-ray exposure. Restraint caused <16 and 29 % decreases in Flt-3L levels following exposure to 6 Gy of X rays at 1 and 2 d post-exposure, respectively. No significant effects of restraints were seen for G-CSF.

Effect of partial-body exposure

The combined use of haematological blood counts and plasma proteomic biomarkers can enhance radiation dose assessment[12]. Preliminary results for the effect of partial-body exposure were earlier reported on lymphocytes, Flt3L and SAA[4]. Here the authors report on the results from additional haematology parameters (i.e. neutrophils—Figure 3B; neutrophil-to-lymphocyte ratio—Figure 3C; platelets—Figure 3D)

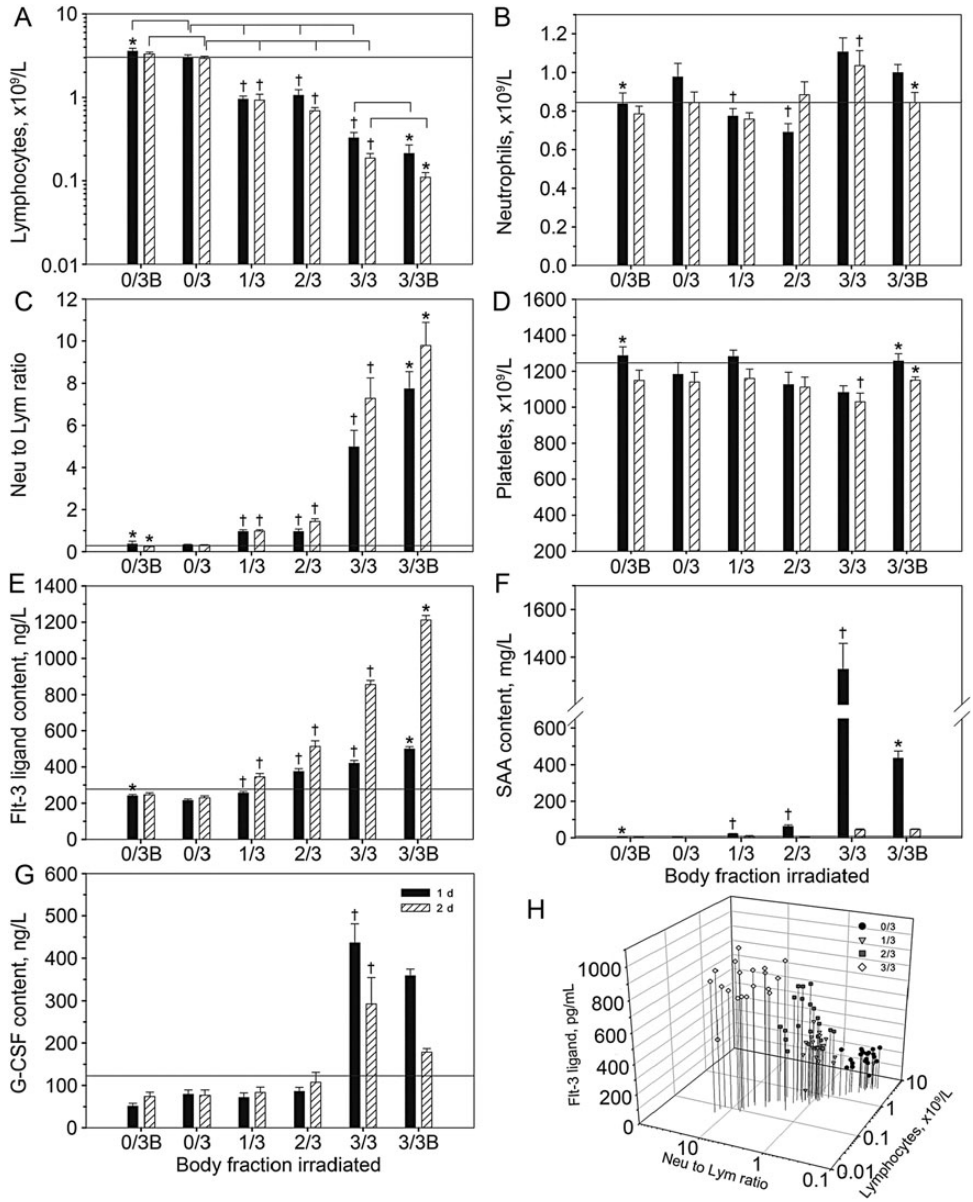

Figure 3. Effects of restraint and partial-body exposure on selected biomarkers. Baseline levels of biomarkers are shown as a horizontal line in (**A**)–(**G**). Baseline data were obtained from Ossetrova *et al.*[24] . Mice were sham treated in the mouse irradiation box (0/0B) and the single-mouse holder (0/0). Restrained mice were exposed to 6 Gy X rays to their mid-body (1/3), lower body (2/3) and whole body (3/3). Non-restrained mice were exposed to 6 Gy X rays (3/3B). Blood was biosampled 1 and 2 d after irradiation for blood cell counts [(A) lymphocytes, (B) neutrophils, (C) neutrophil to lymphocyte ratio, (D) platelets)] and selected plasma protein ELISAs [(E) Flt3L, (F) SAA and (G) C-CSF]. Bar heights represent means ($n = 24$). Error bars represent SE of the means. Statistical differences ($p < 0.05$) in the non-restrained groups compared with the restrained groups are indicated with asterisks (Table 1) and between the exposed restrained and 0 Gy restrained are indicated with crosses. (H) The benefit of using multiple biomarkers to enhance distinguishing of different partial-body exposures is illustrated in a 3D-plot of radioresponse using Flt3L, lymphocyte counts, and neutrophil-to-lymphocyte ratio. This study involved three replicate experiments with $n = 8$ for each experiment. See manuscript text for additional details. Data from the partial-body exposures (i.e. 0/3, 1/3, 2/3 and 3/3) for biomarkers shown in (A), (E) and (F) were previously reported[4].

Table 1. Effect of restraint.

Fold changes in biomarker levels between restrained vs. non-restrained mice

Biomarker	0 Gy		6 Gy	
	Ratio	*p*-Value	Ratio	*p*-Value
Lymphocytes				
1 d	1.28	0.012	1.78	0.001
2 d	1.13[a]	0.14	1.85	0.003
Neutrophils				
1 d	1.21	0.024	1.08[a]	0.33
2 d	1.08[a]	0.25	1.24	<0.001
Neutrophils-to-lymphocyte ratio				
1 d	1.42	<0.01	0.59	0.005
2 d	1.24	0.005	0.70	0.019
Platelets				
1 d	0.93	0.013	0.56	<0.001
2 d	1.00[a]	0.98	0.92	0.031
Flt3L				
1 d	0.90	0.031	0.84	<0.001
2 d	0.93[a]	0.24	0.71	<0.001
SAA				
1 d	1.99	0.026	2.94	<0.001
2 d	ND[b]	—	1.65[a]	0.28
G-CSF				
1 d	1.56[a]	0.11	1.18[a]	0.14
2 d	1.12[a]	0.53	1.57[a]	0.06

[a]No significant effect of restraint in biomarker levels when comparing restrained vs. non-restrained mice based on a two-tail Student's *t*-test ($p < 0.05$).
[b]ND, not done.

and plasma protein G-CSF (Figure 2G) evaluated following exposure to 0 or 6 Gy of X rays.

Both lymphocyte counts and neutrophil-to-lymphocyte ratio showed changes with progressive increases in the fraction of the body exposed to 6 Gy of X rays, whereas neutrophil and platelet count responses were not significantly different with changes in the fraction of the body exposed.

Plasma protein biomarker G-CSF was measured in this model system 1 and 2 d after irradiation. Plasma G-CSF baseline (non-restrained) level ranged from 5.78 to 204.91 ng L^{-1} with a sham-treatment (0 Gy, restrained) mean value of 78.98 (±10.48) and 76.68 (±12.76) ng L^{-1} for 1 and 2 d, respectively. Whole-body exposure to 6 Gy of X rays caused a 5.52 (±0.93) and 3.81 (±1.03) ng L^{-1}-fold increase in G-CSF level at 1- and 2-d relative to the sham controls (Figure 3G). Mice exposed to 6 Gy to either the mid-body (1/3) or mid- and lower-body (2/3) showed a markedly less proportional radioresponse compared with whole-body exposures. By 2 d after radiation, whole-body exposed (3/3 and 3/3B) G-CSF levels lowered but remained well above background levels (Figure 3G), which may be attributed to the importance of head irradiation and involvement of the severe inflammatory response syndrome[14].

Multiple radiation-responsive biomarkers can contribute to enhance assessment of partial-body dose assessment. Three haematological-based biomarkers (Flt3L, lymphocyte counts and neutrophil-to-lympho cyte ratio) were selected to illustrate their combined ability to distinguish partial-body exposure (Figure 3H).

SUMMARY

Radiation model systems that address relevant potential confounders to validate novel biodosimetry strategies are needed. Here the effects of stress due to restraint and partial-body exposure were evaluated using a murine partial-body exposure model. Haematological biomarkers are well accepted for use in radiation dose and injury assessment for medical management of radiological casualties[15] and as demonstrated here for partial-body exposure situations using the murine radiation model. The authors have previously demonstrated their efficacy for radiation diagnostics using non-human primate whole-body exposure radiation models[16, 17].

Similar findings were also demonstrated using a murine whole-body exposure model[12]. Flt3L has been proposed as a bone marrow aplasia biomarker based on studies using animal radiation models[18], radiotherapy patients[19] and human radiation accidents[20–22].

The results have shown support for the use of biomarker Flt3L under confounding conditions of stress due to restraint and partial-body exposures. The combined use of haematology blood count parameters and plasma proteomic biomarkers also lead to a paradigm shift from biomarkers of dose to bioeffects (i.e. bone marrow ARS subsyndrome)[23].

ACKNOWLEGEMENTS

The authors acknowledge the assistance of H.D. Clinton (Veterinary Sciences Department) for haematology measurements and HM2 (SW) S. Gallego for technical contributions. The opinions, conclusions and recommendations expressed or implied do not necessarily reflect the view of the Department of Defense or any other department or agency of the U.S. federal government.

FUNDING

The Armed Forces Radiobiology Research Institute (AFRRI) supported this research under work unit RBB4AR and RAB4AU (awarded to W.F.B.). Biomedical Advanced Research and Development Authority (BARDA), part of the Office of the Assistant Secretary for Preparedness and Response within the U.S. Department of Health and Services also supported components of this research under

Contract No. HHSO100201000009C (MSD) and Inter-Agency Agreement No. AFR.10.064 (AFRRI) project G1B2ET (awarded to N.I.O.).

REFERENCES

1. Prasanna, P. G. S. *et al*. *Synopsis of partial-body radiation diagnostic biomarkers and medical management of radiation injury workshop*. Rad. Res. **173**(2), 245–253 (2010).
2. Durante, M., Yamada, S., Ando, K., Furusawa, Y., Kawata, T., Majima, H., Nakano, T. and Tsujii, H. *Measurements of the equivalent whole-body dose during radiation therapy by cytogenetic methods*. Phys. Med. Biol. **44**(5), 1289–1298 (1999).
3. Hérodin, F. *et al*. *Assessment of total- and partial-body irradiation in a baboon model: preliminary results of a kinetic study including clinical, physical, and biological parameters*. Health Phys. **103**(2), 143–149 (2012).
4. Blakely, W. F., Sandgren, D. J., Nagy, V., Kim, S-.Y. and Ossetrova, N. I. *Murine partial-body radiation exposure model for biodosimetry studies—preliminary report*. Radiat. Meas. **46**, 898–902 (2011).
5. Roy, L., Bertho, J. M., Souidi, M., Vozenin, M. C., Voisin, P. and Benderitter, M. *Biochemical approach to prediction of multiple organ dysfunction syndrome*. Br. J. Radiol. **27**(suppl.), 146–151 (2005).
6. Blakely, W. F., King, G. L., Ossetrova, N. I. and Port, M. *Molecular biomarkers of acute radiation syndrome and radiation injury*. In: Blakely, W. F., Janiak, M. K., Edwards, K. and Duffy, F. (Eds.) Radiation Bioeffects and Countermeasures (Chapter 5, pp: 5–1 to 5–22), Technical Report, North Atlantic Treaty Organization, Research and Technology Organization, Human Factors and Medicine-099 RTO-TR-HFM-099, AC/323(HFM-099)TP/356, Neuilly-sur-Seine, France: RTO. Available on http://www.rto.nato.int (2011).
7. Mossé, I. B. [Modern problems of biodosimetry]. [Article in Russian]. Radiats. Biol. Radioecol. **42**(6), 661–664 (2002).
8. Hanzha, O. B., Rodionova, N. K., Dolyns'ky?, H. A., Kostiukova, L. V. and Druzhyna, M. O. [Non-specific stress-reaction as indicator of radiosensitivity]. [Article in Ukrainian] Fiziol. Zh. **54**(6), 72–78 (2008).
9. Ossetrova, N. I. and Blakely, W. F. *Multiple blood-proteins approach for early-response exposure assessment using an in vivo murine radiation model*. Int. J. Radiat. Biol. **85**(10), 837–850 (2009).
10. Ossetrova, N. I., Sandgren, D. J., Gallego, S. and Blakely, W. F. *Combined approach of hematological biomarkers and plasma protein SAA for improvement of radiation dose assessment in triage biodosimetry applications*. Health Phys. **98**(2), 204–208 (2010).
11. Debad, J. D., Glezer, E. N., Wohlstadter, J. N. and Sigal, G. B. *Clinical and biological applications of ECL*. In: Bard, A. J. (Ed.) Electrogenerated Chemiluminescence. Marcel Dekker (2004).
12. Sigal, G. B., Glezer, E., Kenten, J., Kumar, S., Ossetrova, N. I. and Blakely, W. F. *Biomarker-based radiation-dosimetry diagnostics*. In: The Medical Basis for Radiation-Accident Preparedness. Christensen, D. M., Sugarman, S. L. and O'Hara, F. M., Jr. Eds. Oak Ridge Associated Universities. pp. 375–378 (2013).
13. Blakely, W. F., Salter, C. A. and Prasanna, P. G. *Early-response biological dosimetry–recommended countermeasure enhancements for mass-casualty radiological incidents and terrorism*. Health Phys. **89**(5), 494–504 (2005).
14. Gourmelon, P., Marquette, C., Agay, D., Mathieu, J. and Clarençon, D. *Involvement of the central nervous system in radiation-induced multi-organ dysfunction and/or failure*. Br. J. Radiol. **27**(Suppl.), 62–68 (2005).
15. Dainiak, N. *Hematologic consequences of exposure to ionizing radiation*. Exp. Hematol. **30**, 513–528 (2002).
16. Blakely, W. F., Ossetrova, N. I., Manglapus, G. L., Salter, C. A., Levine, I. H., Jackson, W. E., Grace, M. B., Prasanna, P. G. S., Sandgren, D. J. and Ledney, G. D. *Amylase and blood cell count hematological radiation-injury biomarkers in a rhesus monkey radiation model-use of multiparameter and integrated biological dosimetry*. Radiat. Meas. **42**(6–7), 1164–1170 (2007).
17. Blakely, W. F., Ossetrova, N. I., Whitnall, M. H., Sandgren, D. J., Krivokrysenko, V. I., Shakhov, A. and Feinstein, E. *Multiple parameter radiation injury assessment using a nonhuman primate radiation model—biodosimetry applications*. Health Phys. **98**(2), 153–159 (2010).
18. Bertho, J. M. *et al*. *Level of Flt3-ligand in plasma: a possible new bio-indicator for radiation-induced aplasia*. Int. J. Radiat. Biol. **77**, 703–712 (2001).
19. Huchet, A., Belkacemi, Y., Frick, J., Prat, M., Muresan-Kloos, I., Altan, D., Chapel, A., Gorin, N. C., Gourmelon, P. and Bertho, J. M. *Plasma Flt-3 ligand concentration correlated with radiation induced bone marrow damage during local fractionated radiotherapy*. Int. J. Radiat. Oncol. **57**, 508–515 (2003).
20. Bertho, J. M., Roy, L., Souidi, M., Benderitter, M., Gueguen, Y., Lataillade, J. J., Prat, M., Fagot, T., De Revel, T. and Gourmelon, P. *New biological indicators to evaluate and monitor radiation-induced damage: an accident case report*. Radiat. Res. **169**, 543–550 (2008).
21. Bertho, J. M. and Roy, L. *A rapid multiparametric method for victim triage in cases of accidental protracted irradiation or delayed analysis*. Br. J. Radiol. **82**, 764–770 (2009).
22. Bertho, J. M., Roy, L., Souidi, M., Benderitter, M., Bey, E., Racine, R., Fagot, T. and Gourmelon, P. *Initial evaluation and follow-up of acute radiation syndrome in two patients from the Dakar accident*. Biomarkers **14**, 94–102 (2009).
23. Blakely, W. F. *Recommended approach for use of multiparameter biodosimetry*. In: The Medical Basis for Radiation-Accident Preparedness. Christensen, D. M., Sugarman, S. L. and O'Hara, F. M., Jr. Eds. Oak Ridge Associated Universities. pp. 33–49 (2013).
24. Ossetrova, N. I., Condliffe, D. P., Ney, P. H., Krasnopolsky, K., Hieber, K. P., Rahman, A. and Sandgren, D. J. *Early-response biomarkers for assessment of radiation exposure in mouse total-body irradiation model*. Health Phys. **106**(6), 772–786 (2014).